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Journal of Otology 9 (2014) 151–155

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Induced pluripotent stem cells: Landscape for studying and treating hereditary hearing loss

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Received 16 October 2014; revised 24 October 2014; accepted 19 November 2014

Abstract

Hearing loss (HL) is one of the most widespread sensory disorders, affecting approximately 1 in 500 newborns. Heritable diseases of the inner ear are the leading causes of prelingual HL. Treating of hereditary HL and understanding its underlying mechanisms remain difficult challenges to otolaryngologists. As stem cells are capable of self-renewal and differentiation, they are ideally suited both for disease modeling and regenerative medicine. Recently, description of induced pluripotent stem cells (iPSCs) has allowed the field of disease modeling and personalized therapy to become far more accessible and physiologically relevant, as iPSCs can be generated from patients of any genetic background. This review briefly describes the advantages of iPSCs technology and discusses potential applications of this powerful biological tool in studying and treating hereditary HL.

Keywords: IPSCs; Hereditary hearing loss; Disease modeling; Genetic correction

1. Introduction

Hearing loss (HL) is one of the most common birth defects and prevalent sensorineural disorders, affecting approximately 1 in 500 newborns with bilateral congenital sensorineural hearing loss ≥40 dB HL (Hilgert et al., 2009a). More than two-thirds of prelingual HL cases are attributed to hereditary factors, most of which are caused by mutations of a single gene that functions in the inner ear (Hilgert et al., 2009a; Morton and Nance, 2006). HL can be present in a non-syndromic form (70%), as a single disorder, or syndromic form (30%) associated with distinctive clinical features (Genetics Evaluation Guidelines for the Etiologic Diagnosis of Congenital Hearing Loss, 2002). More than 150 chromosomal loci and at least 80 genes have been identified to result in non-syndromic as well as syndromic forms of HL, and approximately 1000 causing mutations have been described (Hilgert et al., 2009a, 2009b). Due to unclear mechanisms underlying hereditary HL and the intricate microstructure of the inner ear, treatment of hereditary HL is still a huge challenge to otolaryngologists. Stem cells capable of self-renewal and differentiation are ideally suited both for generating disease models and for obtaining the large quantities of cells required for genetic correction and transplantation therapies. Three major types of stem cells have been adopted: embryonic stem cells (ESCs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs). Especially in recent years, significant advances have been made in the research of inherited disease by iPSC technology, opening an avenue to generate patient-specific pluripotent stem cells. With the presence of retroviral integration, human iPSCs are useful in understanding disease mechanisms, drug screening, and regenerative medicine

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Peer review under responsibility of PLA General Hospital Department of Otolaryngology Head and Neck Surgery.

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http://dx.doi.org/10.1016/j.joto.2015.02.001

(Takahashi et al., 2007). The iPSC technology have a broad application for neurodegenerative diseases, blood disorders and retinal degenerative diseases, but little is known for hereditary HL (Wiley et al., 2015; Ross and Akimov, 2014; Wang et al., 2012). In this review, we will briefly describe the advent of iPSCs technology and discuss potential applications of this powerful biological tool in studying and treating hereditary HL.

2. Comparison among three types of stem cells

All three types of stem cells contain two remarkable cellular characteristics that make them ideal candidates for regenerative medicine applications: the properties of "self-renewal" and "pluripotency". Self-renewal refers to the ability of these cells to make identical copies of themselves indefinitely, without developing chromosomal abnormalities or undergoing growth arrest. Pluripotency refers to the ability of these cells to differentiate into any cell of the human body, following the natural path of human embryonic development, when given the appropriate signals.

ESCs are harvested from the inner cell mass of the blastocyst of 5-day-old pre-implantation embryos. As ESCs can differentiate into the three germ layers, they are a popular tool used in regenerative medicine. ASCs are undifferentiated cells, found throughout the body after development that multiply by cell division to replenish dying cells and regenerate damaged tissues. These cells are suggested to be present in small numbers in most major organs, such as bone marrow, skin, brain and inner ear (Davanger and Evensen, 1971; Gage, 2000; Li et al., 2003). ESCs have played a substantial role in disease modeling and treatment studies in the past decades, however, harvesting ESCs from the inner cell mass of the blastocyst during development restricts their clinical practicality due to limited availability and ethical concerns. In addition, ESCs are by definition non-autologous, not derived from the patient for which they are destined, and for cell transplantation an additional obstacle of immune incompatibility exists (Wiley et al., 2015). Although the use of ASCs has no ethical issues and less concerns for immune rejection, limited potency and capacity for self-renewal hinders their further research.

Yamanaka and his colleagues revolutionized the stem cell field in 2006 when they demonstrated that murine fibroblasts could be reprogrammed into ESC-like pluripotent stem cells by transfecting four transcription factors, Klf4, Oct3/4, Sox2, and c-Myc (KOSM), termed induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006). One year later, Yamanaka's group successfully derived iPSCs also from human fibroblasts, thus making his work relevant to human disease and an incredibly promising potential resource for cellular transplantation studies (Takahashi et al., 2007). True pluripotency of iPSCs has been demonstrated by successful production of viable mice from iPSCs through tetraploid complementation (Zhao et al., 2009). However, in conventional transduction, retroviral vectors are randomly integrated into the host's genome, thus significantly increasing the risk of insertional mutagenesis and oncogenesis (Okita et al., 2013). In order to generate clinical-grade iPSCs, safer methods have become the main goal of development in reprogramming technology. The most promising are DNA-free and viral-free protocols. They include introduction of reprogramming-inducing molecules into cells such as: recombinant proteins, messenger RNA (mRNA), and mature microRNA (miRNA) (Kim et al., 2009; Warren et al., 2010; Miyoshi et al., 2011). The efficiency of non-integrating reprogramming methods is significantly enhanced by the use of low oxygen level conditions and small molecules such as histone deacetylase inhibitors and/or DNA methyltransferase inhibitors (Szablowska-Gadomska et al., 2011; Huangfu et al., 2008; Mikkelsen et al., 2008). The traditional methods of obtaining somatic cells requires harvesting skin specimens, which increases patient's discomfort. Some researchers have found that other human somatic cells derived from blood or urine samples also can be reprogrammed into iPSCs, which greatly facilitate their clinical applications (Zhou et al., 2012; Staerk et al., 2010).

The discovery of iPSC ushered in a new age in the field of disease modeling and regenerative medicine. Unlike ESCs, the human iPSCs are not constrained by ethical disputes, and, although not yet fully tested, they should be safer immunologically (Wiley et al., 2015). A key advantage of the iPSC technology is that these cells can be generated in large numbers using cells taken from the patients for which they are intended. Hence, iPSCs are patient-specific. Additionally, like ESCs, iPSCs are pluripotent and can be differentiated into any cell type of the three embryonic germ layers (Takahashi et al., 2007).

3. Contribution of IPSCs to hearing research

3.1. Hereditary hearing loss modeling

Most hereditary HL cases are caused by monogenic mutations (Angeli et al., 2012). They are usually manifested by functional defect of the organ of Corti – a structure of receiving, encoding and transmitting acoustic signals to higher auditory processing stations. Since the discovery of the first nonsyndromic deafness gene in 1993, more than 150 loci of deafness genes have been mapped and more than 80 genes have been implicated in nonsyndromic HL. These genes fall into four broad functional categories: hair bundle morphogenesis, ion homeostasis, extracellular matrix composition and transcription factors (Hilgert et al., 2009a; Yan and Liu, 2008). Spontaneous and induced mouse models of hereditary HL are routinely used for understanding of genesbiological relevance to auditory function. However, producing knockout, knock-in and conditional mutant gene-targeted mice is a high-cost and time-consuming process, especially knockout of certain genes such as BMP4 will cause the mouse to die before the inner ear is formed (Hogan et al., 1994). In addition, such models rarely mirror human disease pathological mechanisms, and the human response is often difficult to be predicted from these models (Blanton et al., 2002). It is assumed that patient-derived iPSCs can replace millions of animals currently

sacrificed in preclinical testing and provide a new route to generate model organism. The first analysis of a disease-specific mutation was that of JAK2-V617F in myeloproliferative disease in 2009 (Ye et al., 2009). Since then, several diseases have been modeled using iPSC technology. For example, patient-specific Alzheimer's disease iPSCs were produced that displayed the differences of pathological markers compared to controls (Israel et al., 2012). Thus, through the generation and analyses of iPSCs from normal subjects and hereditary disease patients, we will improved cellular and molecular understanding of human hereditary HL (Fig. 1).

It is believed that iPSCs are a useful tool for the interrogation of disease pathophysiology in relatively inaccessible tissues such as the inner ear that cannot be routinely subjected to molecular analysis in living patients. Critical point for the ability to model hereditary HL is the capacity to generate inner cells from iPSCs. A study presented a stepwise guidance protocol starting with iPSCs, which were directed toward becoming ectoderm capable of responding to otic-inducing growth factors (Oshima et al., 2010). The resulting otic progenitor cells were plated onto a layer of mitotically-inactivated chicken utricle stromal cells, which promoted the organization of the cells into epithelial clusters displaying hair cell-like cells with stereociliary bundles that were labeled with phalloidin and antibody to espin (Oshima et al., 2010). Bundle-bearing cells in these clusters responded to mechanical stimulation with currents that were reminiscent of immature hair cell transduction currents (Oshima et al., 2010). Another study showed that neurite outgrowth from iPSC-derived neural progenitors toward cochlear hair cells *ex vivo*, and followed their expressed markers for glutamatergic neurons after transplantation into mouse cochleae *in vivo* (Nishimura et al., 2009). Neurons derived from iPSCs projected neurites toward cochlear hair cells (Nishimura et al., 2009). These findings indicate that iPSCs can be differentiated into hair cell-like cells and spiral ganglion neurons, therefore, it is feasible to model hereditary HL by using patient-derived iPSCs.

3.2. Genetic correction and cell replacement treatment

Despite that hearing aids and cochlear implants are relatively satisfactory solution for hereditary HL, future treatment will have to rely on basic rather than symptomatic approaches. A new treatment strategy is to create immunologically-matched and genetically-corrected hair cells or spiral ganglion neurons that can be transplanted into the cochlea to restore hearing. An ideal inner ear cells source of no immune rejection is patient-specific iPSCs, and recent advances in genome editing approaches, including the use of zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered, regularly interspaced, short palindromic repeat (CRISPR)/CRISPR-associated nuclease 9 (Cas9), are especially exciting for the potential boost to genetic correction of cellular level (Christian et al., 2010; Hockemeyer et al., 2011; Miller et al., 2007). The simplicity of design makes CRISPR/Cas9 more promising than the other two genome editing techniques. Components of the prokaryotic CRISPR/Cas9 adaptive immune system have enabled the development of RNA-guided nuclease technology for targeted genome editing (Cong et al., 2013; Maeder et al., 2013). The *S. pyogenes* Cas9 can be directed by a ~100 nt single guide

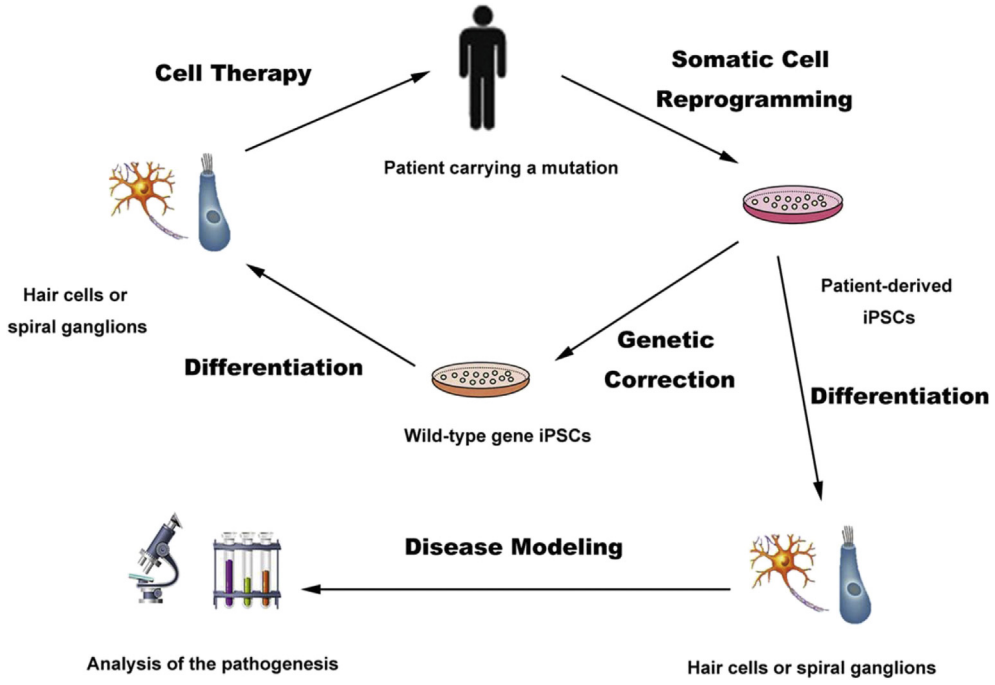

Fig. 1. Schema of how induced pluripotent stem cell (iPSC) can be applied in a clinical setting. These iPSCs may contain a mutation that can be differentiated directly into inner ear cells and transplanted back into the patient to model hereditary diseases of hearing loss or to correct them using gene therapy.

RNA (sgRNA) to a target genomic DNA sequence that is complementary to the first 20 nts of the sgRNA and flanked by a protospacer adjacent motif (PAM) sequence of the form NGG (Jinek et al., 2012). Similarly to ZFN- and TALEN-mediated gene correction, cells repair the majority of CRISPR/Cas9-induced doublestrand breaks (DSBs) by both homologous and non-homologous mechanisms. Applications based on the two DSB repair pathways lead to introduction of different types of mutations at target-specific sites of the genome including gene knock-out, knock-in and point mutations. In the absence of a homologous repair template, the cell naturally employs nonhomologous end joining (NHEJ) leading to the insertion or deletion (InDel) of sequence surrounding the DSB (Sun et al., 2012). Repair via NHEJ represents a feasible strategy for intronic mutations in areas of poorly conserved sequence. However, to correct loci at which large deletions or insertions occur or where NHEJ-mediated InDels would not be tolerated, the sgRNAs and Cas9 must be co-delivered with an engineered repair template containing unmutated wild-type sequence. DSB repair occurs via homologous recombination using the exogenous repair sequence as template. Homology-directed repair (HDR) in diseased patient cells harboring mutations would presumably restore wild-type gene and protein expression and function. The unavoidable off-target effects have largely restrict the application of CRISPR/Cas9 indirectly genome editing of target cells *in vivo*, but iPSC technology provides a large number of candidates which could be used to screen non-off-target cells. Thus, following CRISPR-based correction, iPSCs-derived inner ear cells to be used for human transplantation would no longer harbor disease-causing mutations.

Patient-specific iPSCs technology coupled with CRISPR/Cas9 have the potential to elevate stem cell replacement therapy to being a long-term disease remedy. CRISPR/Cas9 has been shown to precisely correct the dystrophin gene in Duchenne muscular dystrophy (DMD) patient-specific iPSCs (Li et al., 2015). To restore the dystrophin protein, Akitsu Hotta's group performed three correction methods (exon skipping, frameshifting, and exon knockin) by CRISPR/Cas9 in DMD patient-specific iPSCs, and found that exon knockin was the most effective approach (Li et al., 2015). Finally, they differentiated the corrected iPSCs toward skeletal muscle cells and successfully detected the expression of full-length dystrophin protein (Li et al., 2015). These results provide an important framework for developing iPSC-based gene therapy for hereditary HL using programmable nucleases.

4. Future directions

Many exciting developments in the field of iPSC technology present an excellent opportunity to treat hereditary HL and ultimately improve patients' lives. iPSCs can be employed to investigate and determine disease mechanisms from individual patients. Knowledge gained from these experiments can be used to develop gene-based therapies. Transplantation of gene-corrected patient-specific iPSC-derived inner ear cells will treat individuals with hereditary HL. These approaches will be expedited through production of clinical-grade reagents in the near future, so that therapeutics can reach human patients more quickly.

Declaration of conflict of interests

The authors report no competing financial interests.

Acknowledgments

This work was supported by grants from National Basic Research Program of China (the 973 Project, grant numbers: 2012CB967904 and 2012CB967900) and the Cochlear Implantation Program of Hunan, China.

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